



Tools to evaluate processes for oxidase-based biocatalysis

Ramesh, Hemalata; Tufvesson, Pär; Woodley, John

Publication date:
2012

Document Version
Early version, also known as pre-print

[Link back to DTU Orbit](#)

Citation (APA):

Ramesh, H. (Author), Tufvesson, P. (Author), & Woodley, J. (Author). (2012). Tools to evaluate processes for oxidase-based biocatalysis. Sound/Visual production (digital)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Tools to evaluate processes for oxidase-based biocatalysis

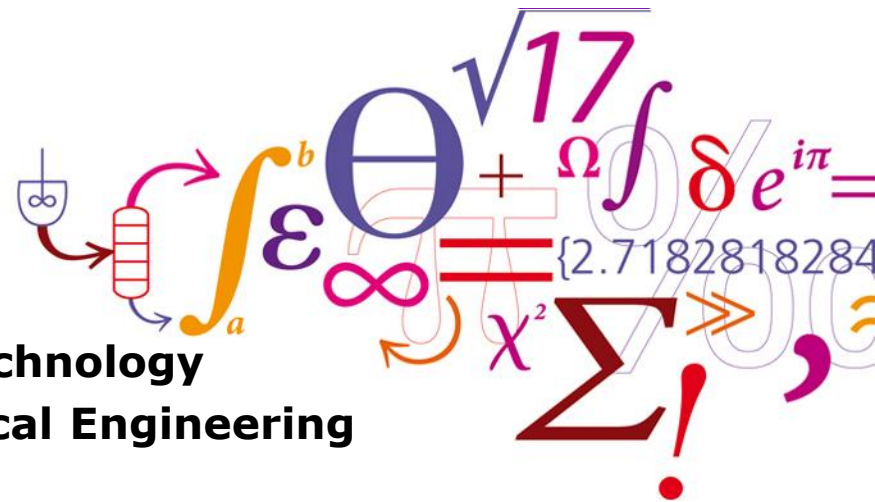
A stability perspective

Hemalata Ramesh

Pär Tufvesson

John M. Woodley

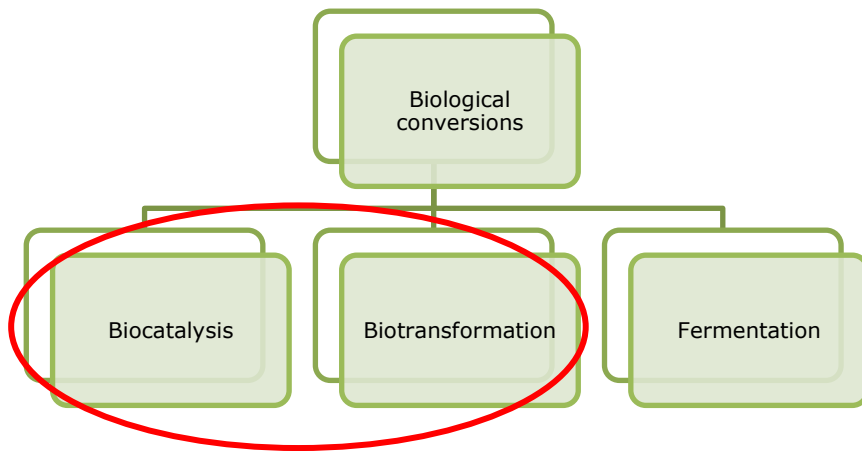
Center for Process Engineering and Technology
Department of Chemical and Biochemical Engineering
Technical University of Denmark



Agenda

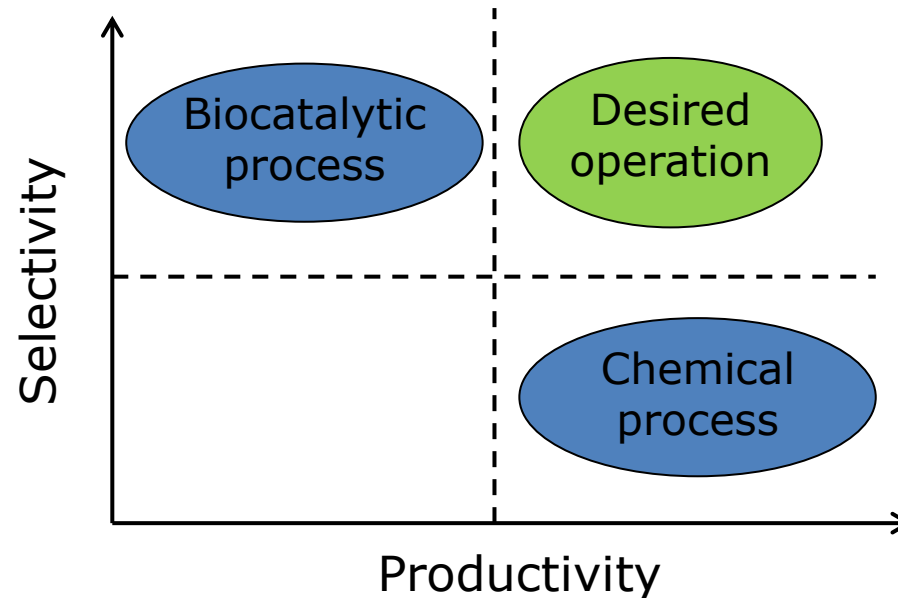
- Introduction
- Challenges involved in oxidase catalysed reactions
- Evaluation of stability issue in a process perspective
- Process tools employed in D-AAO as case study
- Take-home messages

Biocatalysis and Biotransformation



- Selective
- Water based reactions
- Mild conditions
- Catalyst properties can be altered

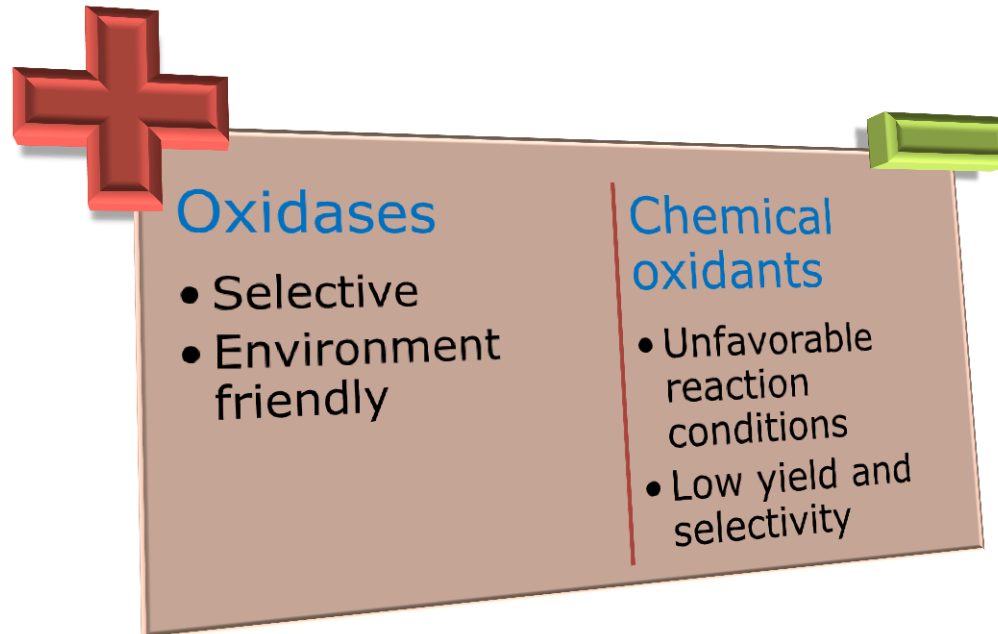
Chemical catalysis and Biocatalysis



Biocatalysis is very different to conventional chemical catalysis –
Different game, Different rules!

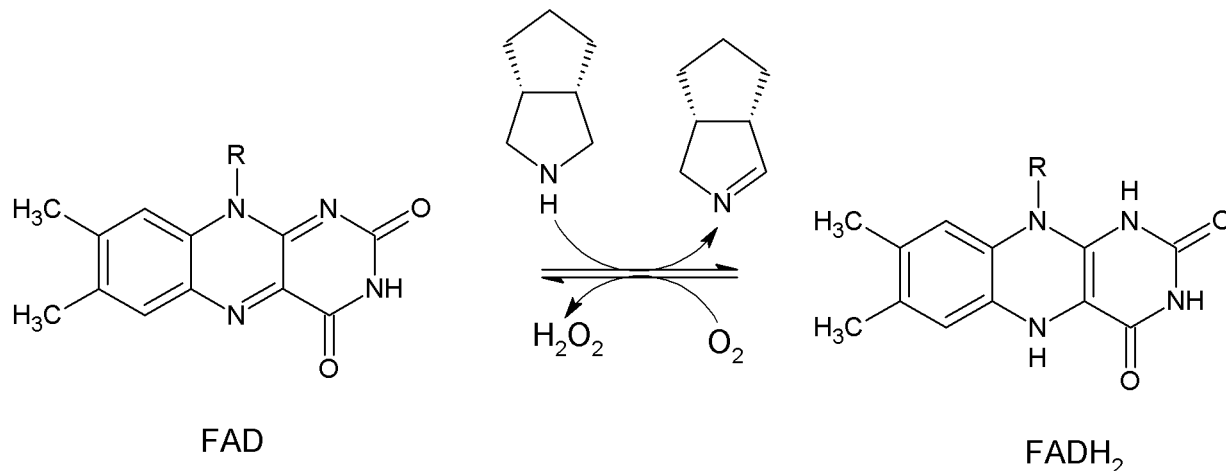
Biocatalysis for oxidation

- Oxidation reactions are key platforms for the development of building blocks for industrially relevant products.



Key reaction scheme of oxidases

- Two important oxidation reactions
 - C-O bond oxidation (alcohol and carbohydrate oxidases)
 - C-N bond oxidation (amine oxidases)
- Test system – A class of amine oxidase called MAO (EC 1.4.3.4)
- Reaction scheme of test system



Challenges

- The reaction scheme for MAO has indicated two key challenges that are associated
 - Supply of oxygen while maintaining stability
 - Hydrogen peroxide inactivation

The challenges are similar to that present in other oxidases, which will be explained further with the help of an example of D-AAO.

Importance of stability

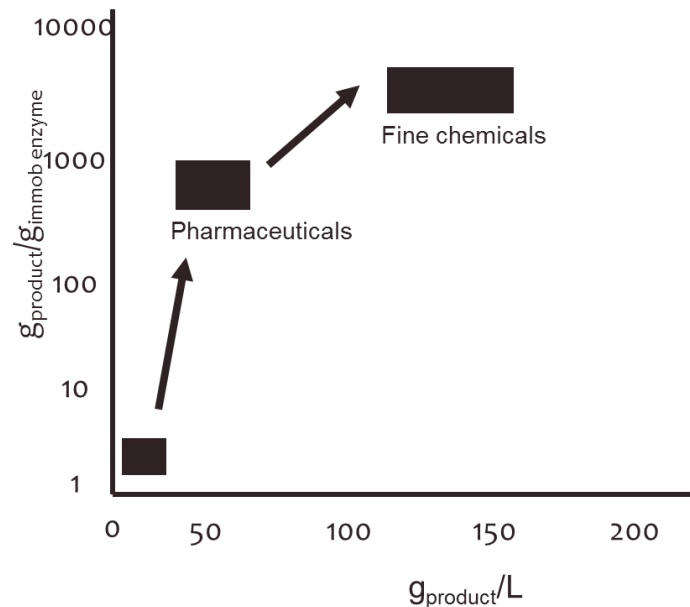


Fig: Productivity targets for different industrial sectors
(Woodley, personal communication)

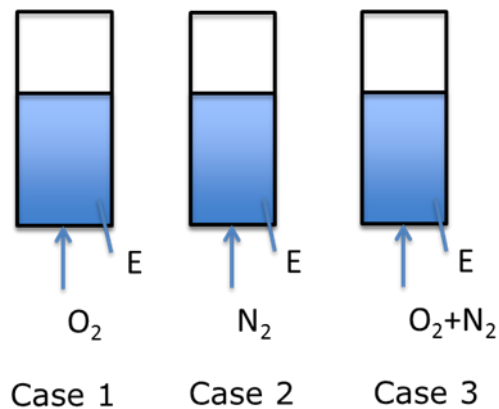
- Depending on the targeted chemical, the required catalyst productivity is depicted in the figure.
- It is inevitable that the biocatalyst be re-used a number of times to reach the productivity whilst keeping the cost down.

Measurement of Stability

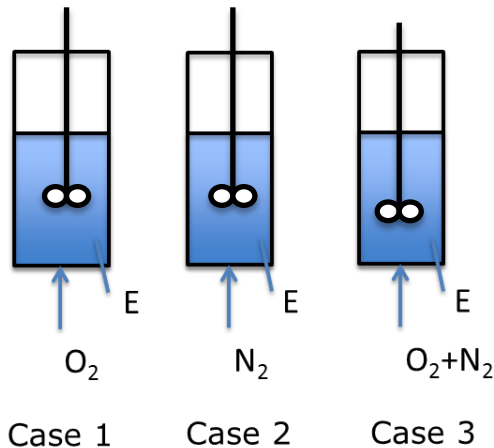
- Typically the stability of a biocatalyst is measured by the number of times it can be re-used in a particular reaction without loss in conversion.
 - Referred to as **operational stability**
- Operational stability is greatly affected by
 - Oxygen
 - Hydrogen peroxide
- In order to study the effect of oxygen on the stability, it is desirable to separate it from contributing effects.

Understanding deactivation by Oxygen

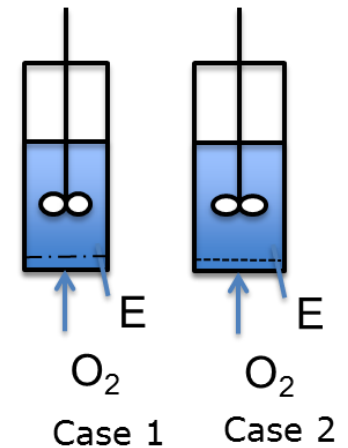
Oxygen concentration



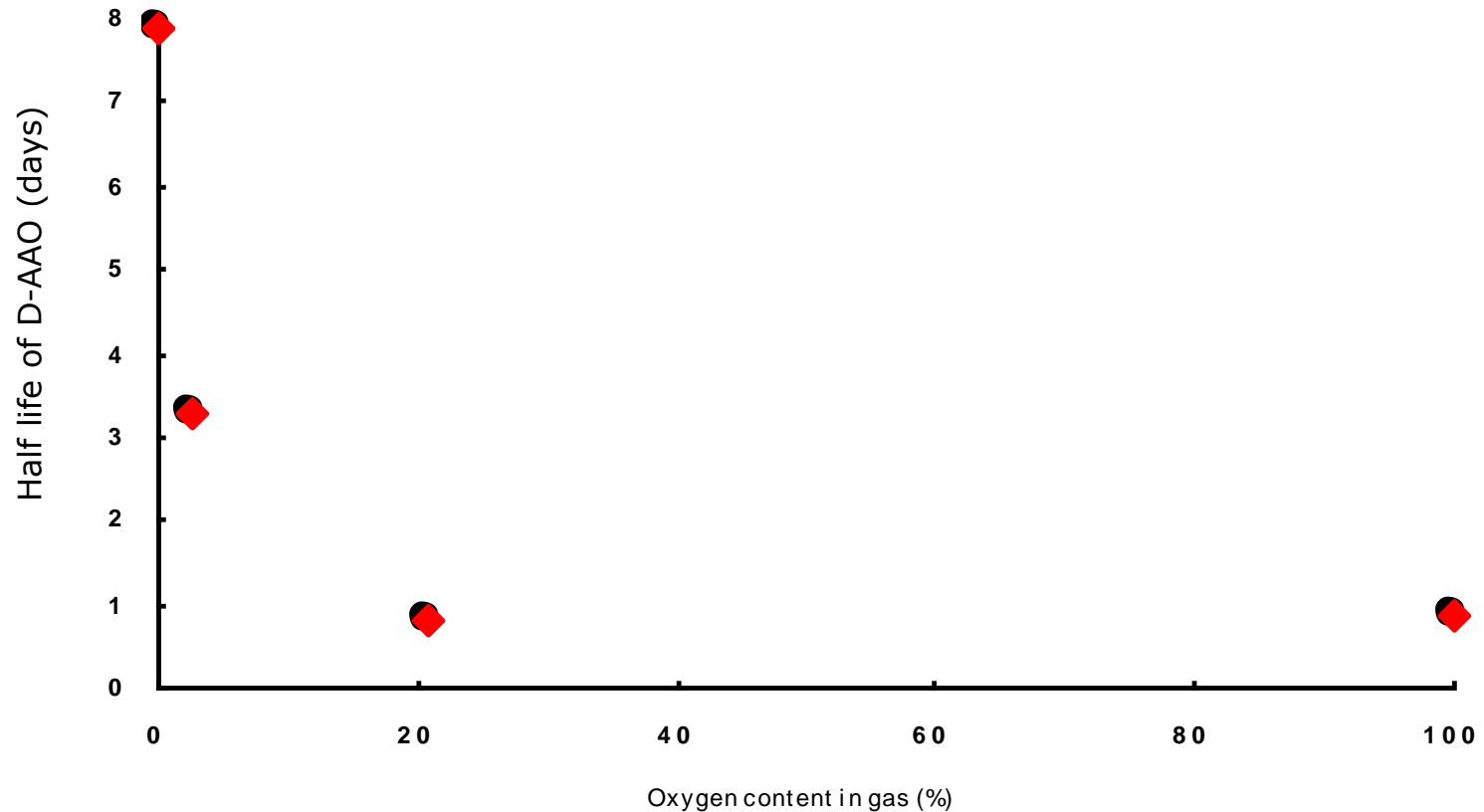
Aeration & agitation



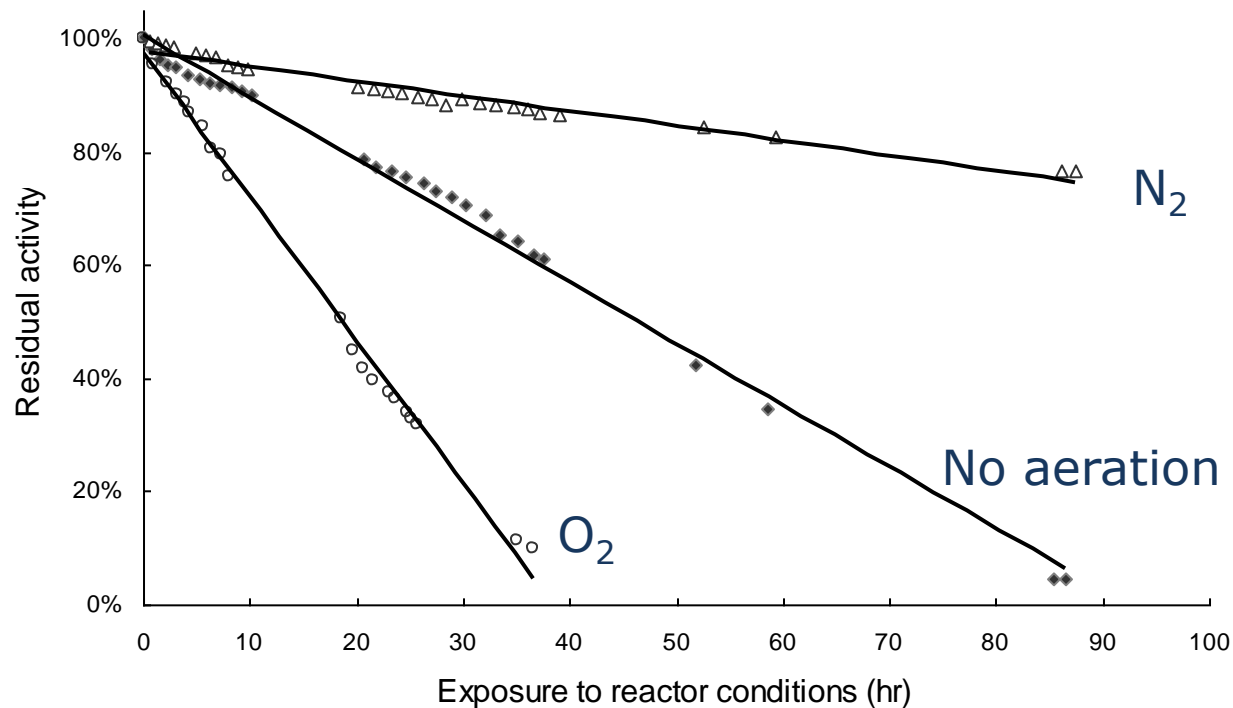
Bubble size



Oxygen concentration on stability

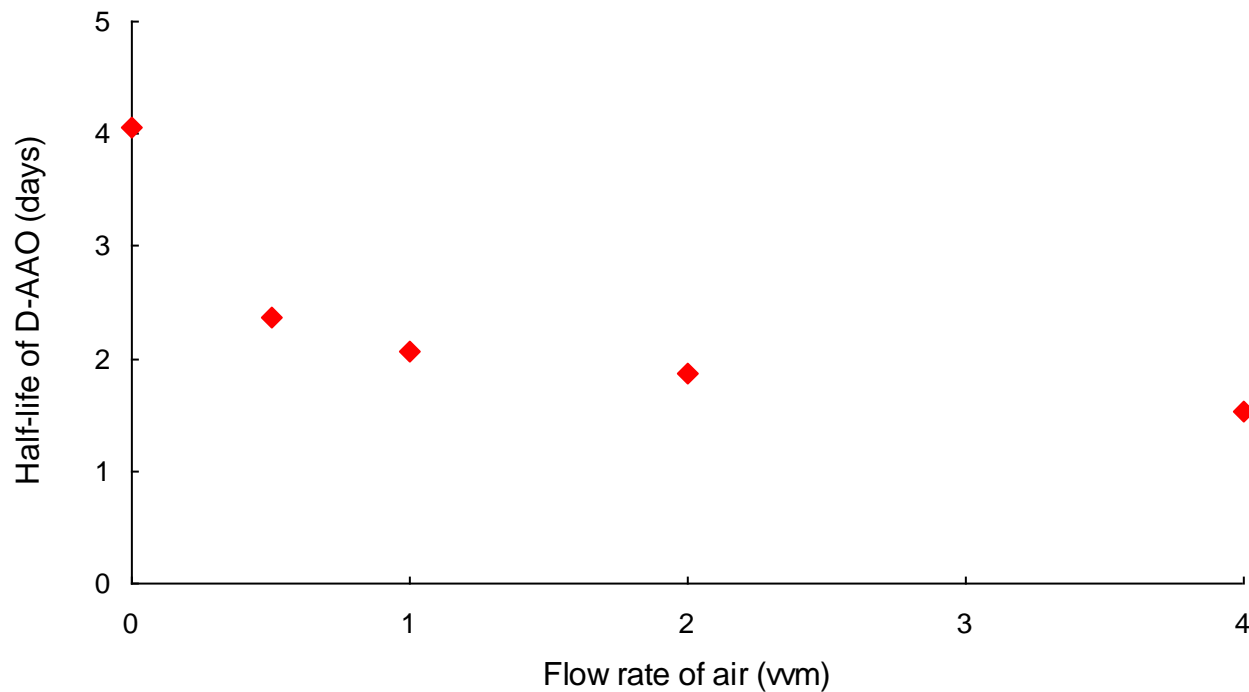


Gas effect on D-AAO stability

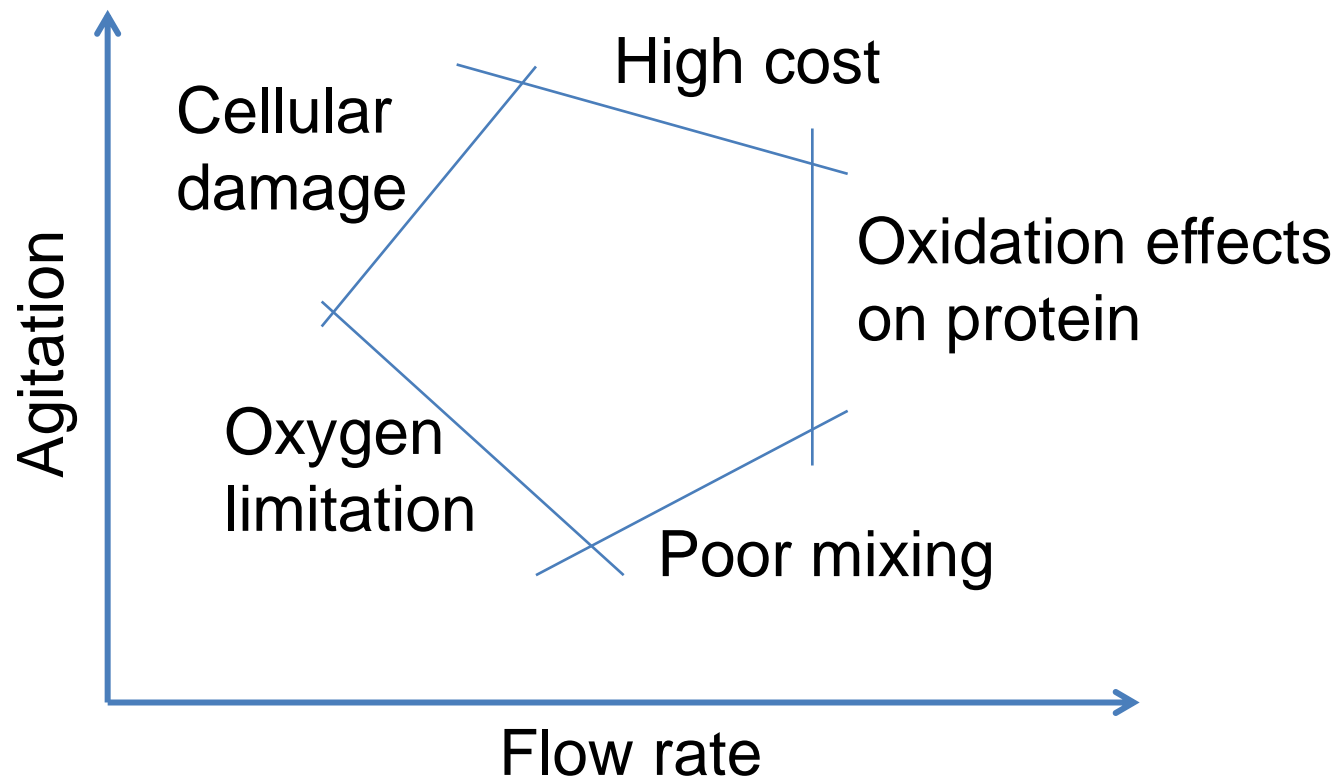


Tindall et. al., 2011

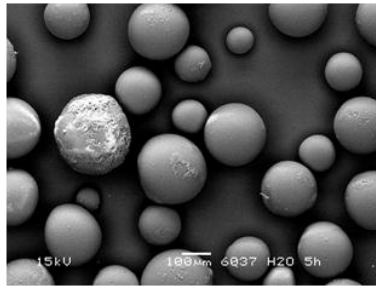
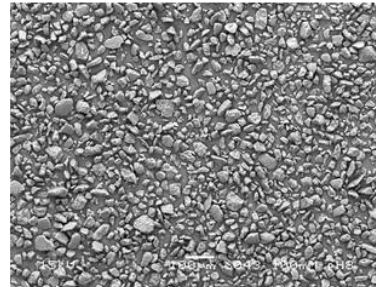
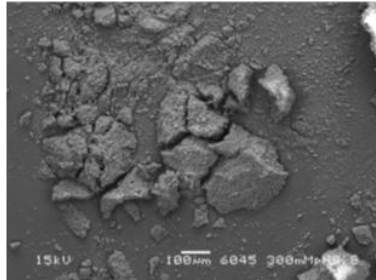
D-AAO stability with flow rates



Operation window



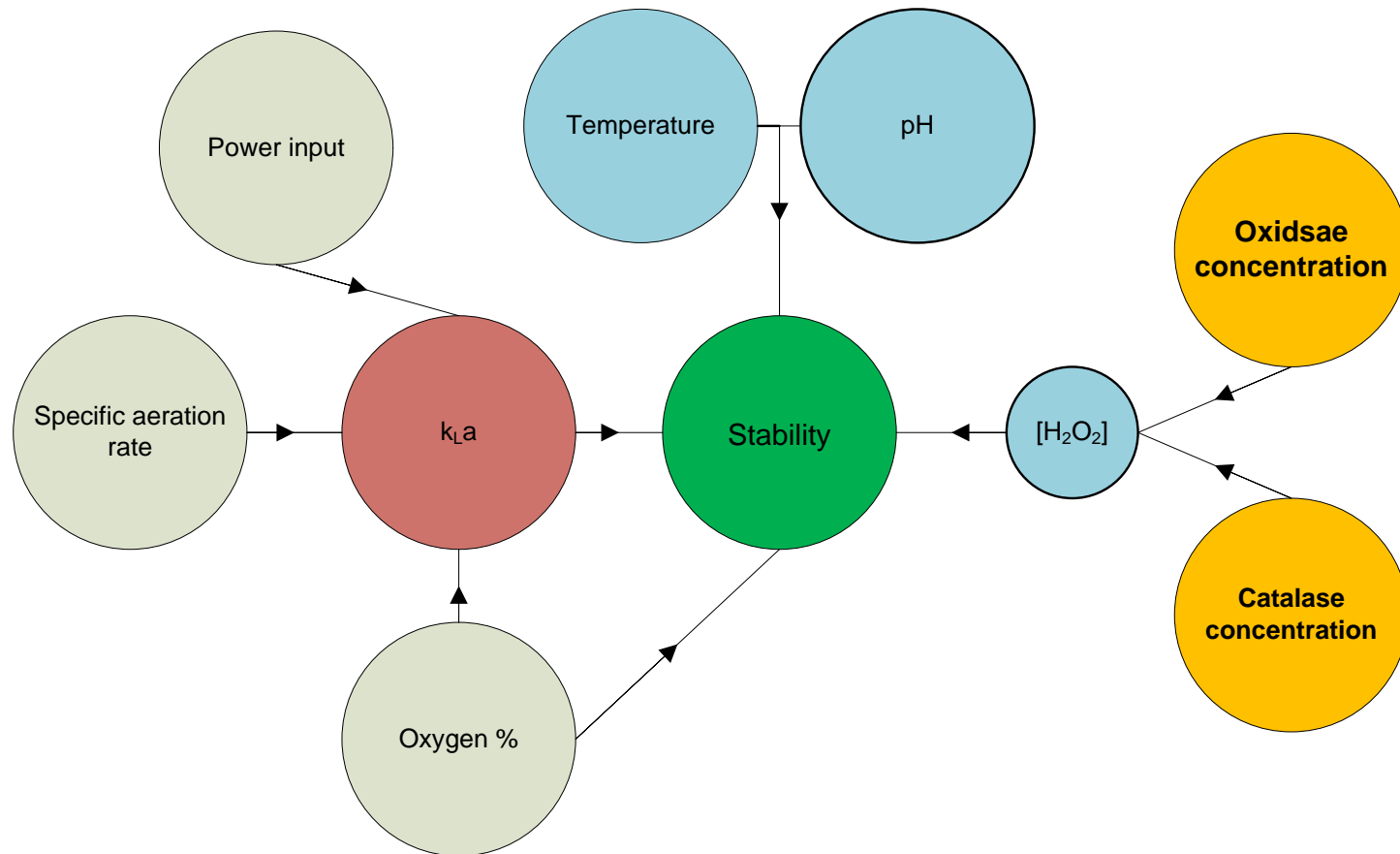
Effect of H₂O₂

$[H_2O_2]$ (mM)	Mean particle diameter (μm)	SEM
0	175-125	
100	37-12	
300	< 5	

Use of know-how in determining the deactivation mechanism

- Operational stability at operating conditions
- While re-use of immobilized catalyst can be made, stability of soluble catalyst can be evaluated by operating a continuous reactor and monitoring the conversion.

Effects contributing to stability of oxidases



Take home messages

- Oxygen effects on biocatalyst must be evaluated independently to understand the direct effects.
- Operational stability is of key importance for the success of a biocatalytic process and stability studies must be incorporated fairly early and go hand-in hand with process development.
- It is critical that the catalyst is evaluated under process conditions using scale-down strategy.
- The understanding gained through the studies can be used to develop operation windows which will aid as tools for process development.

Acknowledgements

- BIONEXGEN - UT acknowledges the support of BIONEXGEN, financed through the European Union 7th Framework Programme (Grant agreement no.: 266025)
- SSCHE
- PROCESS
 - John M. Woodley
 - Ulrika Törnvall
 - Pär Tufvesson
 - Mathias Nordblad
 - Stuart R Tindall

Contact: hemra@kt.dtu.dk

Thank you!

